REMARKS

Courtesies extended to Applicants' representative in the personal interview held on October 29, 1999, are acknowledged with appreciation.

The present invention is based on the discovery that recombinase nucleic acid constructs can be expressed at high levels in the germ line but not to a functionally significant extent in either ES cells or embryonic or adult somatic tissues.

Accordingly, the present invention provides transgenic embryonic stem (ES) cells containing recombinase responsive nucleic acid constructs (claims 12-15, 18-24, and 26) useful for preparation of transgenic animals, such as mice. The transfected nucleic acids comprise a germ-line promoter (other than ZP3) operatively linked to a recombinase encoding gene. The ES cells transfected with such nucleic acids can further contain a transcriptionally active selectable marker flanked by recombinase recombination sites and/or a nucleic acid fragment flanked by recombinase recombination sites specific for a different recombinase than the recombination sites that flank the selectable marker, and/or a nucleic acid construct comprising an inducible promoter operatively associated with a recombinase coding sequence, or a tissue-specific promoter operatively associated with a recombinase coding sequence (claim 15). Because the germ line promoter directs recombination events in the germ line, but only to a de minimus amount in other tissues, embryos can be derived from such ES cells that contain a transgenic allele, such as is caused by homologous recombination at recombinase target site(s).

In addition, when the ES cells contain a nucleic acid fragment flanked by recombinase recombination sites specific for a different recombinase than the recombinase expressed in the germ line (i.e., different than the recombination sites that

flank the selectable marker), such as under control of an inducible or tissue specific promoter, recombination of the target site can be controlled to occur in a specific (i.e. somatic) tissue or in an inducible manner. In addition, because the ES cells contain a germ-line specific promoter operatively linked to the recombinase encoding gene, the transcriptionally active selectable marker can be excised by passage of the genome derived from said embryonic stem cells through gametogenesis. ES cells obtained by crossing the genome of the transgenic gamete with a wild type genome can be used to obtain ES cells in which the transgene is stably incorporated, into the genome, but the selectable marker is excised. Excision of the marker without excision of the allele of interest allows any phenotype that is observed to be more confidently ascribed to the mutation of interest rather than to some combination of that mutation and the transcriptionally active marker.

For the creation of tissue-specific or inducible mutations, site-specific recombinases are used in an initial step to excise the marker, and, in combination with a tissue-specific or inducible recombinase transgene, to subsequently excise some essential component of the target of interest in the intact animal.

In accordance with the invention there are also provided methods for the production of recombinant mice (i.e., containing recombinant alleles), as well as methods for conditional assembly of functional genes for expression in eukaryotic cells by recombination of individual inactive gene segments from one or more genes of interest using the invention ES cells containing recombinase responsive nucleic acids.

Claims 1-10, 12-16, 18-26 and 28-45 were pending before this response. By the present communication, claims 1-10, 16, 25, 33, and 45 have been canceled and claims 12, 18-24, 26, 28, 32, 35, 40, 43, and 44 have been amended to define Applicants' invention with greater particularity. Therefore, claims 12-15, 18-24, 26, 28-32, and 34-44 remain pending. These

amendments add no new matter as the amended claim language is fully supported by the specification and original claims. It is respectfully submitted that the proposed amendments submitted herewith would place the claims in condition for allowance or at least in better condition for appeal; accordingly, entry of the amendments is respectfully requested.

The Rejection Under 35 USC § 112, First Paragraph

Applicants respectfully traverse the rejection of claims 1-10, 12-16, 18-26 and 28-44 for alleged lack of enablement under 35 USC § 112, First Paragraph. Applicants disagree with the Examiner's assertion that:

> the specification clearly demonstrates obtaining expression of a marker gene in the brain, spleen and heart tissue at low levels. Applicants argue the expression obtained in spleen and heart tissue is minimal and that the expression in testes is over 100 times greater. ... The bottom line is that marker genes are expressed in brain, spleen and heart tissue of mice at low levels using the instant invention. The specification does not enable one of skill to determine whether the expression of any gene is functionally significant in the instant invention. ... the production of a ProCre mouse alone is not of use because it does not have a phenotype that distinguishes it from the wild-type mouse ... expression of a marker gene is not of apparent use as disclosed in the specification.

(Office Action, pages 3-4.) With regard to the tissue specificity of Applicants' germline promoters, Applicants submit that it is well known in the art that the term "tissue-specific promoter" encompasses promoters that produce certain low levels of activity in tissue other than the target tissue (In fact, Applicants define the term "tissue specific" as referring to "the substantially exclusive initiation of transcription in the tissue from which a particular promoter, which drives expression of a given gene is derived" Specification, page 8, lines 15-18). Therefore the Examiner's assertion that the specification fails to enable use of a "germ line-specific" promoter is not consistent with the art-understood meaning of the term.

Regarding the alleged failure of the Specification to enable one of skill to determine whether the expression of any gene is functionally significant in the instant invention (Office Action, page 3), as Applicants have pointed out in the previous response, the Specification teaches that the level of ectopic activity of the recombinase gene operatively linked to a "germline specific promoter" in the heart, brain and spleen in all transgenic individuals tested was "more than 100 fold lower than that observed in testis" (Specification, page , line 24). In addition, the Specification teaches:

> Activities observed in other strains [of mice] were considerably lower than this, and one strain [strain 75] did not show any ectopic activity.

(Specification, page 17, lines 30-32; emphasis added). Thus, Applicants respectfully submit that the Specification teaches that expression obtained in "other tissues," such as the atrium of the heart, that is at least 100 times less than in testes, is not "functionally significant."

Applicants further disagree with the Examiner's assertion that the embodiment of the invention transgenic animal illustrated in the Specification (i.e., a transgenic mouse that expresses a marker gene) "is not of use" because the mouse does not have a phenotype. Applicants respectfully submit that the utility of the invention transgenic mouse and the invention transgenic ES cells used to produce the mouse would be readily understood by those of skill in the art as a research tool useful for studying the effect of knocking out any gene targeted by the invention recombinase recombination mechanism contained in the transgenic mouse.

The function and reliability of recombinase recombination for disrupting a target gene was well understood in the art at the filing date of the present application, as is shown by the cited art and as acknowledged by the Examiner in the Office Action (see the quote from Office Action, page 10). Applicants, therefore, wish to direct the Examiner's attention to the exact mechanism that Applicants illustrate in the Specification, i.e., the use of a germline specific promoter to trigger

activity of an operatively associated recombinase coding sequence in ES cells such that a nucleic acid flanked by two responsive recombinase recombination target sites under homologous recombination in ES cells during gametogenesis and is excised from the germline in first generation animals obtained from such ES cells. Furthermore, a second nucleic acid encoding a different recombinase and which is operatively associated with a promoter that is not activated during gametogenesis becomes integrated into the genome of the ES cells during gametogenesis. What gene will be targeted by the recombinase recombination sites is the choice of the researcher who will use Applicants' invention research tools.

Furthermore, the value of such research tools lies in the information obtained by using them and not simply and solely in the creation of a "phenotype" in a transgenic animal. In fact, valuable research information could be obtained from the failure to create a phenotype in a transgenic mouse in an experiment utilizing an embodiment of Applicants' invention wherein any particular functional gene is substituted in the place of the marker gene or the P2Bc gene Applicants used to illustrate the invention. Applicants respectfully submit that those of skill in the art would readily understand this concept and the utility of the invention research tools.

Therefore, Applicants specifically disagree with the Examiner's assertion that:

> embodiments encompassing ES cells used to make mice expressing marker genes are not enabled because such cells have no disclosed use.

(Office Action, page 3). Applicants respectfully submit that the Specification fully illustrates utility of the invention ES cells as a research tool or as a tool of manufacture for conveniently producing transgenic animals, such as mice, that are functionally wild-type but can contain heterologous nucleic acid constructs in their genomes. It is up to the researcher to determine what phenotype is to be studied using the invention ES cells and mice prepared using the ES cells.

In analysis of utility under 35 U.S.C. §101 or 35 U.S.C. §112, first paragraph, it has been determined that: "When a properly claimed invention meets at least one of its stated objectives, utility under §101 is clearly shown." (*Roper v. Raytheon*, 742 F.2d 951, 220 USPQ 592, 598 (Fed Cir. 1984)). The Examiner appears to be taking the position that, because an invention is useful to researchers, it is unpatentable. This, of course, is not the standard that was established by the U.S. Supreme Court's opinion in *Brenner v. Manson* (383 U.S. 519, 148 U.S.P.Q. 689 (1966). In *Brenner v. Manson*, the Supreme Court affirmed that "no patent be granted on a chemical compound whose *sole* 'utility' consists of its potential role as an object of use-testing" (383 U.S. 519, 148 USPQ 689, 696 (1966); emphasis added). Specifically, in *Brenner v. Manson*, the *only* asserted utility for the claimed compound was its use as a human therapeutic. Before such use could be accepted, further use-testing was required. Therefore, the Supreme Court concluded that the compound's *sole* utility was as an object of further testing to determine a use.

As illustrated in the Examples of Applicants' Specification, a selectable marker or another nucleic acid of interest can be excised during gametogenesis from Applicants' ES cells. Such ES cells are useful to study the effect of deletions upon an embryo or for production of a gamete that is functionally wild type when crossed with a wild type genome but will transfer to the genome of the offspring a heterologous allele. Prior to initiation of a site-specific recombination of the target gene, the offspring is functionally wildtype (i.e., has no observable phenotype). But the invention transgenic mouse contains the cellular machinery that will allow a researcher to excise all or part of a target locus. If the excision disrupts a critical part of the gene, a null allele is created. No similar research utility (i.e., non-therapeutic utility) was alleged in *Brenner v. Manson.*

Thus, Applicants respectfully submit that the invention ES cells and transgenic animals themselves have a known function and are valuable reagents for researchers, not merely *objects of* further research.

Not only is the utility of these ES cells and the transgenic mice produced using them both definite and in a currently available form, but a "real world value" can be placed on them. The commercial market for such ES cells and transgenic animals provides clear evidence of such value.

Moreover, U.S. Patents issue regularly featuring reagents used in research. Just a few notable examples are Mullis *et al.* U.S. 4,687,195; Mullis *et al.* U.S. 4,683,202; and Gelfand *et al.* U.S. 4,889,818, featuring PCR reagents, kits and techniques, which are used as investigatory tools to amplify DNA. The rejection in this case is equivalent to a decision that the PCR technique is not useful under the patent statute because no utility has been shown for the DNA it can amplify. The U. S. Patent and Trademark Office recognizes the patentable utility of reagents and methodologies used in such investigations. Applicants respectfully submit that to allow and issue some patents on research tools while denying others is arbitrary and capricious.

In addition, regarding Applicants' enablement of methods for producing a lethal allele using the invention constructs and methods, Applicants disagree with the Examiner's assertion that:

> Applicants arguments are not persuasive because the level of chimerism required to allow survival and transmission of a transgene is not taught in the specification and because the specification does not teach how to "mask" lethality by cross-breeding with a wild-type animal.

(Office Action, pages 4-5). Applicants respectfully submit that those of skill in the art would understand that the "masking" of lethality happens automatically in a certain percentage of the animals obtained. Thus, no teaching in the Specification regarding the "level of chimerism" or "how to 'mask' lethality" is required for those of skill in the art to practice the embodiment of the invention wherein a lethal allele is obtained.

Moreover, Applicants respectfully disagree with the Examiner's assertion that the illustration of the invention using mouse ES cells and the production of transgenic mice from such ES cells fails to teach those of skill in the art how to practice the invention in other species of animals (Office Action, page 5). In particular, the Examiner asserts that "the isolation of ES cells in species other than mice was unpredictable in the art at the time of filing" (Office Action, page 5). In the prior Response herein, Applicants have traversed the Examiner's argument that the practice of the invention in mice as illustrated in the Specification cannot be extrapolated to other species. That argument is incorporated herein by reference. However, in order to reduce the issues and expedite prosecution, by the present communication Applicants have proposed amendments to all claims to focus the invention on the embodiment wherein the ES cells are mouse cells and the transgenic animal is a transgenic mouse.

In view of the amendments proposed herein and the above arguments, Applicants submit that all pending claims are fully enabled by the teachings of the Specification. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

The Rejection Under 35 U.S.C. § 112, Second Paragraph

The rejection of claims 33, and 40-42 under 35 U.S.C. § 112, Second Paragraph, for alleged indefiniteness is respectfully traversed.

With regard to claim 33, Applicants respectfully disagree with the Examiner's assertion that the term "essential" lacks definition in the specification and in the art, resulting in alleged indefiniteness. However, it is respectfully submitted that the phrase "an essential portion of a gene of interest" as used in claim 33 is not indefinite because those of skill in the art would understand that an "essential" portion of DNA is a portion of the sequence that is required to produce a biological function of the DNA in the cell, whether the DNA encodes a functional

protein or transcription regulatory regions, or whether the DNA is part of an intron whose function cannot be determined. Stated another way, those of skill in the art would understand that deletion of a portion of DNA that is not "essential" would not interfere with the native biological function of the DNA or its protein product in the cell or organism. Furthermore, Applicants submit that those of skill in the art using Applicants' invention as a research tool would know how to determine when an "essential" portion of DNA has been excised.

However, to expedite prosecution and reduce the issues on appeal, by the present communication, Applicants have canceled claim 33, thus removing the grounds of the rejection for alleged indefiniteness as to claim 33.

Applicants respectfully disagree with the Examiner's assertion that the phrase "recombinase responsive construct" in claim 40 is indefinite. The issue has been rendered moot by Applicants' deletion of the phrase from claim 40 in the previous Response mailed herein on June 14, 1999.

Applicants respectfully disagree with the Examiner's assertion that claim 44 is unclear because it "does not result in a step wherein the recombinant livestock (with a non-wild type phenotype) occurs" (Office Action, page 4). The Examiner's attention is directed to the fact that claim 44 has been amended by the present communication to focus prosecution on the embodiment of the invention methods wherein the transgenic animal produced is a mouse. Accordingly, claim 44 has been amended to add the requirement "such that a recombinant mouse is thereby produced," thereby expressly requiring production of the recombinant mouse.

In view of the amendments and the above remarks, Applicants respectfully submit that claims 33, 40 and 44 are definite under 35 U.S.C. §112, Second Paragraph.

The Rejection Under 35 U.S.C. § 103

I. The rejection of claims 1-2, 4-5, 10, 12-16 18-19 24-26 and 28-44 for alleged obviousness over Gu et al. (Science :265-1-3-106, 1994, hereinafter "Gu") in view of Zambrowicz *et al.* (*Biology of Reproduction 50*:65-72, 1994, hereinafter "Zambrowicz"), and Lakso et al. (Proc. Natl. Acad. Sci, 93:5860-5865, 1996) is respectfully traversed. Applicants disagree with the characterization of the combination of the references presented in the Office Action, which states:

> Applicants arguments are not persuasive because the combined teachings of Gu et al., Zambrowicz et al. and Lakso et al. teach all the limitations of the claims, provide motivation to combine the references and provide a reasonable expectation of success in making and using the claimed invention. The system taught by the combined teachings of Gu et al., Zambrowicz et al. and Lakso et al. is a Cre-lox system with a sperm-specific promoter. This system may be used to excise the marker gene from the ES cells as claimed. ...motivation to combine is provided by Lakso et al. who suggest limiting the extended culture of ES cells when exposing ES cells to recombination by directing recombination to the embryo ... The addition of the sperm-specific promoter taught by Zambrowicz et al. to the vector taught by Gu et al. directs recombination to the embryo and limits the time the ES cells are in culture.

(Office Action, pages 8). Applicants invention, as defined by the present claims, distinguishes over the teaching of the cited art, whether taken alone or in combination, by requiring mouse embryonic stem cells containing a nucleic acid construct comprising a germline-specific promoter selected from the group consisting of the protamine 1 gene promoter, the protamine 2 gene promoter, the spermatid-specific promoter from the c-kit gene, the sperm-specific promoter from angiotensin-converting enzyme, the oocyte specific promoter from the ZP1 gene, and oocyte specific promoter from the ZP2 gene operatively associated with a recombinase coding sequence, wherein the nucleic acid construct is in the genome of the stem cells.

Applicants' invention, as defined by claim 12, further distinguishes over the cited art by requiring transgenic mouse embryonic stem cells containing two nucleic acid constructs, one with a transcriptionally active marker gene under the control of a germ-line specific promoter flanked by recombinase recombination sites, and one having a polynucleotide target flanked by recombinase recombination sites. If the nucleic acid fragment is a transcriptionally active selectable marker, such mouse embryonic stem cells can be isolated using the marker.

Applicants further teach that development of such mouse ES cells through gametogenesis and crossing of the transgeneic gamete with a wild type mouse gamete results in a germ-line from which transgenic mice can be obtained having an allele that results from deletion of the marker gene in the germline, as required by claim 40. Applicants have shown that an efficiency of as high as 92% recombination of the target allele can be achieved using this method "Of 112 target alleles transmitted by males of all 5 lines, 103 or 92%, were recombined." (Gorman, page 14604, Col. 2, top). Applicants further teach that unless the marker gene and its transcriptional sequences are excised in the germline of the transgenic offspring, any phenotype detected in the offspring is not confidently ascribed to the presence of the allele because in some cases an interfering effect has resulted from the gene encoding the marker.

If the ES cell used to obtain offspring contains recombination sites that are specific to two different recombinases, such as Cre and FLP, one can be used to trigger deletion of the marker during gametogenesis, and the other can be used later to remove the flanked target DNA to create a transgenic allele in the offspring. Applicants have shown that loxP-flanked targets remain and are not recombined in the somatic tissues of mice that contain ProCre transgenes, but that more than 90% of the progeny sired by these males inherit a Cre-recombined target.

By contrast, Gu fails to disclose mouse ES cells containing a nucleic acid construct comprising a germline-specific promoter operatively associated with a recombinase coding sequence or ES cells further containing a transcriptionally active selectable marker flanked by

two recombinase recombination target sites. Instead Gu discloses a method that requires DNA containing three loxP sites, two of which flank the selection marker genes for neomycin resistance (Neo) and HSV-tk while the second and third flank regions of a germ line target gene (polβ). Cells with homologous recombination which are ganciclovir-resistant because of co-integration of the loxP site are selected. The neo gene is deleted in an in vitro step. Then, in the second step, such ES clones are transiently transfected with plasmid encoding Cre under control of a T-cell specific promoter so that a recombination event occurs only once in some of the transfected ES cells. By this procedure, about 40% of the cells selected in the first cloning step achieve a type I recombination resulting in creation of an allele.

Alternatively, Gu discloses creation of and mating between two transgenic mouse strains, one in which a Cre transgene is expressed in a cell-type specific manner (in T-cells) and a second one in which a transgene flanked by two loxP recombination sites is carried. Presumably, the desired null allele would be found in no more than about 40% of the offspring of such a mating using the method of Gu (page 105, third column, bottom). In summary then, Gu discloses that a two step process, involving two separate cloning steps, is required to achieve a transgenic ES cell carrying an desired allele or a transgenic animal having a desired allele. In particular, Gu teaches that two transgenic animals must be created to obtain offspring bearing the desired mutation in the germ line, requiring much time, and expertise because two separate procedures are required. Based on this analysis, Applicants submit that the present invention provides a substantial improvement over Gu's methods.

Gu is completely silent regarding use of a germ-line specific promoter operatively associated with a recombinase gene so that the recombinase gene is expressed only in germ-line tissue. Thus, Gu discloses no advantage for substantially germ-line specific expression of a recombinase, for example, in creation of a transformed gamete from which ES cells can be obtained wherein the transcriptionally active selectable marker is excised and/or for further creation of a transgenic allele using such ES cells.

Zambrowicz does not overcome all of these deficiencies in the disclosure of the primary reference. Zambrowicz discloses that testis nuclear proteins bind only to a specific region of the testis-specific Prm-1 promoter so as to regulate spermatid-specific transcription and, more specifically, "so as to recognize sequences within and immediately adjacent to a CRE-like sequence" (page 70, Col. 1 bottom, to Col. 2 top). However, Zambrowicz fails to disclose or suggest placing the testis-specific promoter into ES cells for any purpose. Thus, like Gu, Zambrowicz is silent regarding methods for using the combination of any germ-line promoter in a nucleic acid construct containing a gene that encodes Cre, or any other germ-line promoter in a nucleic acid construct, to obtain insertion or deletion of a target gene into embryonic stem cells. Moreover, Applicants respectfully submit that Zambrowicz fails to disclose or suggest Applicants' method, as defined by claims 35 or 40, for obtaining an offspring from a mouse embryonic stem cell wherein the marker gene has been deleted so that the mouse appears to be perfectly wild-type until recombination is triggered via an inducible or tissue specific promoter in a nucleic acid construct having recombinase recombination sites flanking a target nucleic acid fragment.

Applicants specifically disagree with the Examiner's assertion that the motivation to combine the Cre-loxP system of Gu with the M1 promoter taught by Zambrowicz is provided by Lakso et al. citing a passage in which Lasko observes that "excision of the neo expression cassette from a targeted gene removes a functional promoter and a selectable gene product after they have served their purpose . . . to prevent these elements from exerting potentially unwanted effects on the targeted tissue" (page 5865, Col 1 first full paragraph). Applicants respectfully submit that Lakso's statement, in the context in which is appears in the reference, is no more than the expression of a desire for something that does not exist. Lakso does not suggest any method by which such a result could be obtained. For example, Lakso does not in any way suggest how to excise the neo-expression cassette, much less removal of the neo expression cassette in the germ line of ES cells. In fact, Lakso's statement testifies to the existence of a long

felt need in the art for a solution to a problem that others have been unable to solve. Applicants respectfully submit that a reference that testifies to the existence in the art of a long felt need to which a solution is not known, is not the proper standard to be used in selecting a reference to show "motivation" for combining two references that otherwise could not be said to yield Applicants' invention.

Moreover, based on the foregoing analysis of the references cited, Applicants believe that in formulating the rejection for alleged obviousness the Examiner has erroneously relied upon hindsight reconstruction of the disclosure of the references in light of Applicants' teachings. It is well settled in patent law that the Examiner is not allowed to selectively pick and choose elements or concepts from the various references so as to arrive at the claimed invention using the claims as a guide. Hindsight is not a proper criteria for resolving the issue of obviousness. Thus, Applicants further traverse the rejection for alleged obviousness on the grounds that the prior art is being read in light of the teaching of the present specification.

In view of the above amendments and remarks, Applicants respectfully submit that *prima facie* obviousness of claims 1-2, 4-5, 10, 12-16, 18-19, 24-26, and 28-44 is not established by the Gu-Zambrowicz-Lakso combination of references. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

II. The rejection of claims 1, 6-9 and 20-23 for alleged obviousness over Gu in view of Zambrowicz and Lakso as applied in Section I above and further in view of Onouchi *et al.* (*Mol. Gen. Genet* 247:653-660, 1995, hereinafter "Onouchi") is respectfully traversed. Applicants disagree with the characterization of the combination of the references in the Office Action, which states:

> In this case, motivation to combine Gu et al., Zambrowicz et al. and Lakso et al. with Onouchi et al. is provided by Onouchi et al. who state the Cre-lox, FLP-FRT and R-RS system are all similar and cause recombination . . . Thus, one of ordinary skill would have recognized the systems were interchangeable and would have realized a reasonable expectation of success in replacing the Cre-lox system with the FLP-FRT or R-RS system since such genetic manipulations were common at the time of filing.

(Office Action, page 10). Applicants repeat here with respect to claims 1, 6-9 and 20-23 the discussion presented concerning the insufficiency of the Gu-Zambrowicz-Lakso combination of references for disclosing or suggesting the invention nucleic acid constructs and their use in methods of making transgenic stem cells and/or transgenic animals derived from such stem cells having a transgenic allele.

With regard to the assertion in the Office Action that Onouchi motivates substitution of the FLP-FRT or R-RS system and the MP1 promoter to obtain expression of a marker gene, Applicants respectfully submit that the disclosure of Onouchi does not overcome the deficiencies of the Gu-Zambrowicz-Lakso combination of references discussed above. For instance, Onouchi does not disclose ES cells containing a nucleic acid construct comprising an FLP-FRT or R-RS system, or a method of excising a marker gene from the germ line of an ES cell having a nucleic acid construct with a target sequence flanked by recombinase specific recombination sites integrated into the germ line. Nor does Onouchi disclose the obtaining of a first generation transgenic mouse from such an ES cell by crossing the genome of the ES cell with a wild type genome.

Accordingly, Applicants respectfully submit that the combined disclosures of Onouchi, Gu, Zambrowicz, and Lakso fail to teach or suggest the subject matter of present claims 1, 6-9 and 20-23. Accordingly, reconsideration and withdrawal of this rejection are respectfully requested.

Conclusion

In view of the above amendments and remarks, reconsideration and favorable action on claims 1-10, 12-16, 18-26, and 28-45 are respectfully requested. In the event any matters remain to be resolved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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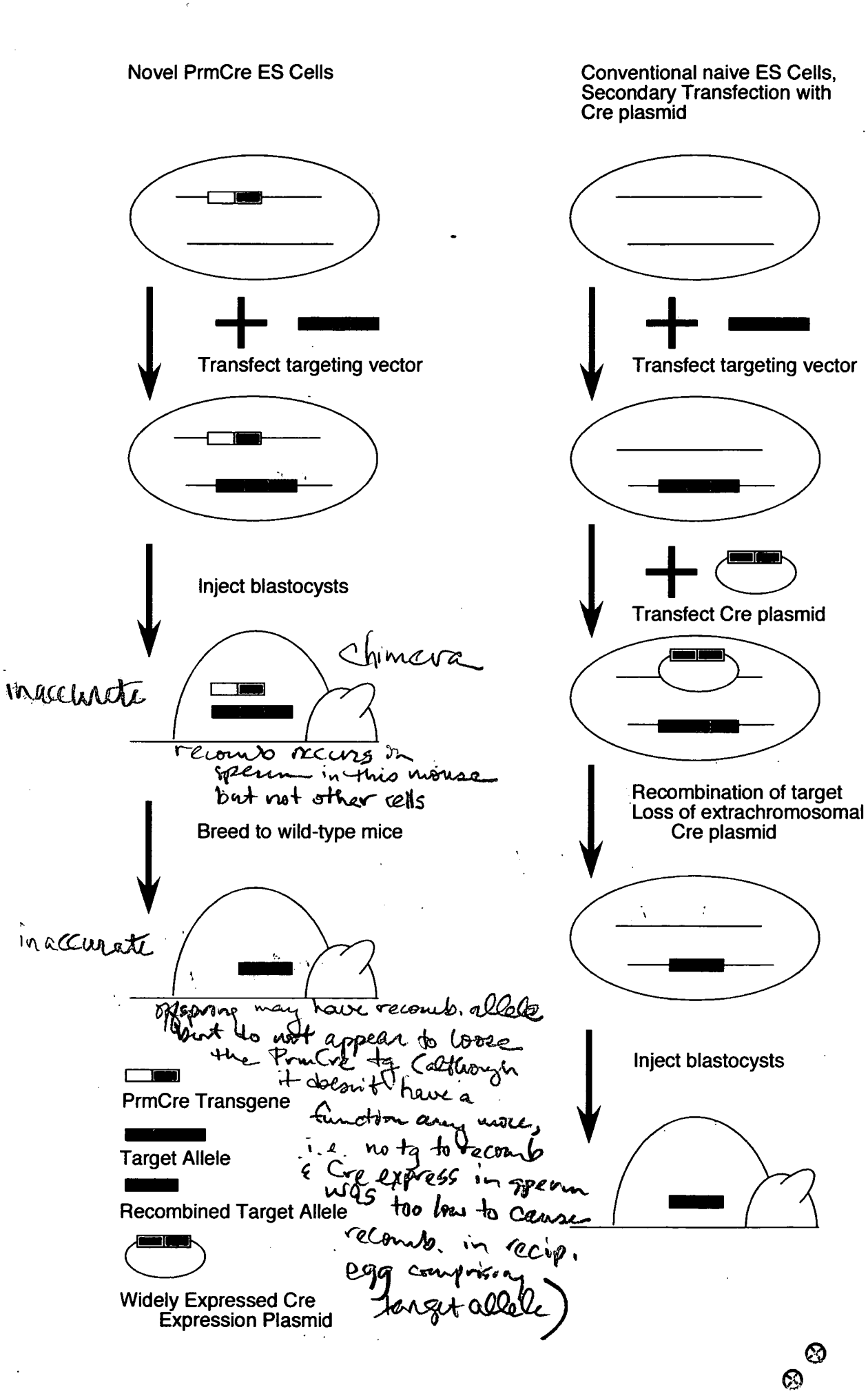
Novel PrmCre ES Cells
Conventional naive ES Cells, Secondary Transfection with Cre plasmid
Transfect targeting vector
Transfect targeting vector
Inject blastocysts
Transfect Cre plasmid
chimera
inaccurate
recomb occurs in sperm in this mouse but not other cells
Recombination of target
Loss of extrachromosomal Cre plasmid
Breed to wild-type mice
inaccurate
offspring may have recomb. allele but do not appear to loose the PrmCre tg although it doesn't have a function any more, i.e. no tg to recomb & Cre express in sperm was too low to cause recomb. in recip. egg containing target allele)
Inject blastocysts
PrmCre Transgene
Target Allele
Recombined Target Allele
Widely Expressed Cre Expression Plasmid